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Transmission of *Helicobacter* spp. A challenge to the dogma of faecal-oral spread

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SUMMARY

Faecal oral spread is claimed by many to be the mode of transmission of the gastric pathogen *Helicobacter pylori*. This idea is based not on experimental data but because the epidemiology of *H. pylori* infection resembles that of other pathogens known to be spread by the faecal-oral route. This is in spite of the observation that no-one has been successful in culturing *H. pylori* from human stool. In this study, a series of transmission experiments are reported on animals infected with the gastric spirilla, *Helicobacter felis* and '*Gastrospirillum hominis*'. Germfree mice and rats infected with *H. felis* did not transmit their infection to uninoculated mice despite prolonged contact in the same cage nor could the bacterium be isolated from their intestinal contents. This was confirmed in specific pathogen free mice where infected dams did not pass the helicobacter to their progeny. Similarly, mice infected with a human isolate of '*Gastrospirillum hominis*' did not transmit the infection while in close contact with uninoculated mice. In contrast, in a limited series of experiments, both *H. pylori* and *H. felis* were transmitted from infected gnotobiotic Beagle puppies to uninfected animals in the same enclosure. In addition, the gastric mucus from a cat with indigenous '*Gastrospirillum*'-like organisms was infectious for mice, whereas faecal content from the same animal was not. It is suggested that the difference between the murine and canine experiments is that the dogs are more likely to have oral-oral contact than rodents. Unlike dogs, mice and rats do not vomit and are coprophagous. It is concluded that the case for faecal-oral spread of *Helicobacter* species is 'not proven' and that the inter-oral route is more likely.

INTRODUCTION

Helicobacter pylori, the spiral shaped bacterium first cultured in Perth, Western Australia nearly 10 years ago is now accepted as the cause of chronic gastritis in humans [1] and as an aetiological agent in the pathogenesis of most peptic ulcers particularly duodenal ulcer [2]. More controversial but potentially of greater import is the suggestion that long term colonization with this organism potentiates

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gastric carcinoma. In certain developing countries such as Columbia and China, *H. pylori* is acquired early in life. The resultant chronic gastritis progresses to atrophic gastritis with ablation of gastric glands and consequent loss of parietal cell function. The hypothesis has been put forward that the ensuing hypochlorhydria leads to gastric overgrowth by various enteric bacteria. The latter in conjunction with dietary factors, produce carcinogenic nitrosamines which induce gastric cancer [3]. If this scenario is confirmed, intervention strategies resulting in the prevention of colonization by *H. pylori* could dramatically alter the morbidity and mortality of gastric carcinoma in these countries.

A fundamental requirement for understanding any infectious disease is an appreciation of the epidemiology, in particular the common route of transmission. Certainly, there is good evidence to support person-to-person spread of *H. pylori*. Families of infected children have a higher incidence of infection [4] and occupants of homes for intellectually handicapped children have significantly higher infection rates than the normal population [5]. Gastroenterologists who conduct endoscopies and are thereby in contact with gastric secretions have a higher infection rate than aged matched groups of other physicians [6]. Many claim that the normal route of spread is faecal oral. The most energetic proponent of faecal-oral spread is Graham, who draws parallels with other enteric infections known to be transmitted by this route, in particular hepatitis A (HAV). He states that the age specific frequency of anti-HAV antibody in a population provides a reasonable gauge of faecal-oral exposure and predicts the age specific rate of onset of *H. pylori* infection in that population, i.e., rapid,. intermediate or slow [7].

There are problems with the faecal-oral hypothesis. No-one has been successful in culturing *H. pylori* from faeces. There is no other example of a proven faecal-oral pathogen that has not been demonstrated in large numbers in a viable form in the stools. Proponents of the faecal route explain this anomaly by drawing on the known characteristic of *H. pylori* to ball up as the culture ages and assume coccal forms [8]. With no evidence, it is suggested that these are resistant forms that pass through the stools in a non-culturable but viable form to be later ingested and cause infection [9].

Lack of convenient animal models of *H. pylori* infection makes the testing of infectivity of stools from *H. pylori* infected patients difficult. The culture by us of a related gastric bacterium with many similar properties to *H. pylori* has made transmission studies possible. This spiral shaped organism was first cultured from the stomach of a normal cat and has subsequently been found in both cats and dogs [10]. Physiological studies and 16s ribosomal RNA studies have shown this bacterium to be a *Helicobacter* sp. and it has been named *Helicobacter felis* sp. nov. [11]. Like *H. pylori*, *H. felis* also produces coccal forms (Fig. 1). Feeding experiments have shown that *H. felis* will colonize the stomachs of both germfree and specific pathogen free (SPF) mice. Another spiral bacterium also seen in cats and dogs is the bacterium tentatively called '*Gastrospirillum hominis*' by McNulty and Dent [12] who first demonstrated it in the gastric mucosa of patients undergoing endoscopy [13]. Since that time human infection with this agent has been reported from many parts of the world. Although this bacterium cannot be cultured *in vivo* we have managed to maintain it *in vivo* by feeding human gastric biopsy specimens to mice [14]. Heavy colonization of the rodent gastric mucosa is

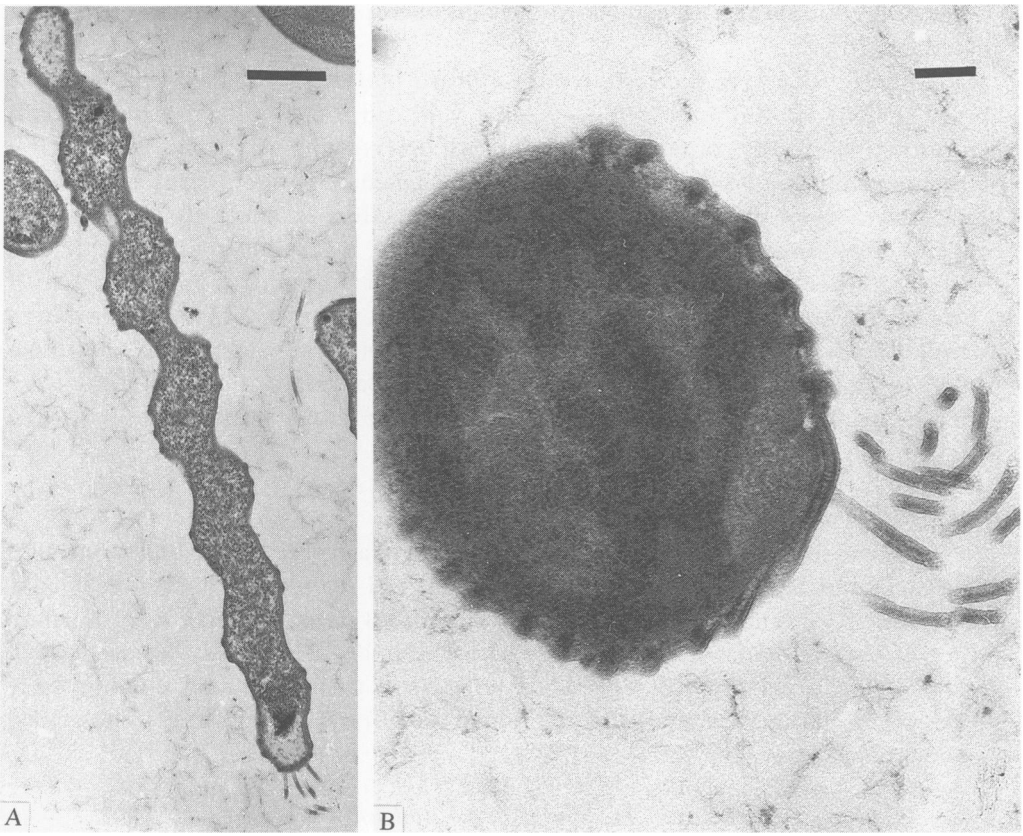

Fig. 1. Transmission electron micrograph of an ultra thin section of cultures of *H. felis*. (A) Fresh culture showing the tight spiral morphology and characteristic periplasmic fibrils. Bar = 1 μm. (B) Coccal form of *H. felis* seen in aged cultures. Bar = 0·1 μm.

achieved and '*Gastrospirillum hominis*' can be transmitted from mouse to mouse by oral inoculation of gastric homogenates. '*Gastrospirillum*', like the helicobacters, is also urease positive and assumes coccal forms in suspension. Given the similarity of these bacteria and gastric infection with them to *H. pylori* infection in humans, transmission of these organisms should be similar. Having two models of gastric infection, we decided to investigate spread of these animal helicobacters. Results of these experiments show that faecal-oral spread does not occur despite the coprophagous habits of rodents.

MATERIAL AND METHODS

Transmission of H. felis *in gnotobiotic and specific pathogen free rodents*

Bacteria inocula

Helicobacter felis (Strain CS1. ATCC 49179), a spiral bacterium originally isolated from cat stomach was grown on blood agar consisting of lysed horse blood (5%) in Blood Agar Base No. 2 (Oxoid) supplemented (final concentrations) with the following antibiotics: vancomycin 10 μg/ml (Sigma), trimethoprim lactate 5 μg/ml (Sigma), polymyxin B 3 μg/ml (Sigma) and amphotericin 2·5 μg/ml

(Fungizone; Squibb) for 48 h at 37° under microaerophilic conditions as previously described [10].

Cells of *H. felis* were harvested from culture plates into brain heart infusion broth (Difco) containing 30% glycerol and adjusted to an approximate concentration of 10^{10} organisms per ml. All harvesting was done with sterile equipment and in a laminar flow cabinet. All suspensions were cultured on sheep blood agar aerobically and anaerobically to ascertain that no bacterial or fungal contaminants were present. While these plates were being incubated, the suspensions were kept frozen at −70 °C. Each test animal was inoculated with 0·2 ml of suspension into the mouth/stomach via a polyethylene catheter cut to 30 mm. The transmission control animals were kept for the same period of time but were not inoculated. In the gnotobiotic experiments, culture vials were sprayed on the outside with peracetic acid before transfer into the germ-free isolators.

Demonstration of infection by H. felis

Animals were killed with carbon dioxide. Stomachs were removed under aseptic conditions and 2 mm square portions were rubbed over lysed horse blood agar plates which were incubated microaerobically for 3 days at 37 °C. Similar small pieces of gastric tissue were also tested by the microtitre biopsy urease test of Hazell and co-workers [15]. Specimens of gastric tissue from all animals were processed for histopathology and detection of infection by microscopy as described previously [16].

Transmission between H. felis *infected and uninfected adult rodents*

Gnotobiotic mice and rats

Four-week-old female Swiss Webster (Tac:(SW) f) isolator reared axenic mice and Tac:N (SD) rats were obtained from Taconic Inc, Germantown, NY, USA. Both mice and rats were maintained in Reynier's type stainless steel isolators at the Forsyth Institute for Dental Research, Boston, MA, USA. All materials were sterilized by pressurized steam or peracetic acid and animals were fed an autoclaved pelleted diet and given sterile water *ad libitum*. The protocols described below were approved by the Animal Care Committees of the Massachusetts Institute of Technology and The Forsyth Institute of Dental Research as well as the Committee for the Use of Animals in Research and Teaching at the University of New South Wales.

As part of a previously reported experiment looking at the histopathology of colonization of germfree mice with *H. felis*, three uninoculated control mice were placed in the same cage as three *H. felis* infected mice for 8 weeks [16]. In a similar experiment, three uninoculated rats were in contact with three infected animals for 8 weeks [17]. The results are shown in Table 1. No transmission occurred from the heavily infected animals to the controls.

SPF animals

BALB/C mice were supplied from the Specific Pathogen Free Production Unit of the Animal Breeding and Holding Unit of the University of New South Wales (UNSW-SPF). CD1 mice were obtained from Charles River Breeding Laboratories,

Table 1. *Transmission between* H. felis *infected rodents and uninfected cage mates*

	Presence of *H. felis* after 8 weeks in same cage	
	H. felis inoculated	Uninoculated
Gnotobiotic mice	3/3	0/3
Gnotobiotic rats	3/3	0/3
SPF CD1 mice	3/3	0/3
UNSW-SPF mice	20/20	0/20

Table 2. *Transmission of* H. felis *between infected mothers their mates and offspring in a single case*

	Number of *H. felis* positive animals	
	28 days	84 days
Infected mothers	—	20/20
Uninfected fathers	0/5	—
Uninfected young	—	0/96

Massachussets, USA. Both these strains of mice were classified as being free of specified microbial pathogens and had been reared under barrier conditions. The natural microbial flora of these animals did not possess any bacteria that normally colonize the stomach.

In a first experiment in Boston, three *H. felis* infected CD1 mice were placed in the same cage as three uninfected mice for 8 weeks. In the second experiment done in Sydney a larger cage was used and 20 adult *H. felis* infected UNSW-SPF mice were mixed with 20 uninfected mice for 8 weeks. Results are shown in Table 1. Once again there was no transmission between the mice despite prolonged and very close contact.

Transmission between H. felis *infected female SPF mice and their male partners and litters*

Twenty adult UNSW-SPF female mice were colonized with *H. felis* and mixed with five uninfected males in a large cage. Four weeks after contact and prior to the birth of litters, the males were removed and assessed for *H. felis* colonization. The 96 offspring resulting from the matings were left in the cage with their dams for 12 weeks. At the conclusion of the experiment all female mice and their litters were assessed for *H. felis* colonization. The results are shown in Table 2. All dams were shown to be heavily colonized with *H. felis*, however, the *H. felis* had not transmitted to any of the males despite 4 weeks of close contact and none of the 96 young had acquired the bacterium even after 12 weeks close contact with their infected mothers.

Transmission of 'Gastrospirillum hominis' *infected CD1-SPF mice and uninoculated cage mates*

'*Gastrospirillum hominis*' (ATCC 49286) originally isolated from a 32-year-old male in Sydney was maintained in the stomachs of mice by serial passage. Infection was carried out as described previously [14]. The contents were removed

Table 3. *Transmission of* 'Gastrospirillum hominis' *and* Torulopsis pintolopesii *between infected and uninfected mice*

	Number of animals positive after 28 days in same cage			
	Inoculated mice		Uninoculated mice	
	Gastrospirillum	*Torulopsis*	*Gastrospirillum*	*Torulopsis*
Transmission experiment 1	3/3	3/3	0/3	3/3
Transmission experiment 2	3/3	3/3	0/3	1/3

from the glandular region of three stomachs of infected mice. The tissue was homogenized in 3 ml of normal saline with a hand held sterile glass homogenizer. Each mouse was inoculated as above with 0·2 ml of homogenate as described above for *H. felis*.

In two separate experiments CD1 mice were infected with the gastric homogenates of Australian mice containing '*Gastrospirillum hominis*' and the natural murine yeast *Torulopsis pintolopesii*. This yeast, which is easily visible on the glandular gastric mucosa is the normal flora of the conventional mice which were used to first isolate '*Gastrospirillum hominis*' [14]. The CD1-SPF mice did not have this yeast as part of their normal flora; thus whenever CD1 mice were colonized with the '*Gastrospirillum*' via stomach homogenate the yeast transferred as well. These inoculated mice were put in a cage together with a number of uninoculated mice. Results are shown in Table 3. There was no transmission of '*Gastrospirillum hominis*' from the infected to uninfected animals despite very close contact in the same cage for 4 weeks. In contrast the murine yeast *Torulopsis* transferred from the infected animals to four of six uninoculated contact mice.

Transmission of Helicobacter *spp. between infected and uninfected gnotobiotic Beagle puppies*

A litter of gnotobiotic Beagle pups (seven in each litter) were derived from specific pathogen free bitches by standard methods [18]. They were maintained in sterile Pentub isolation units and fed a diet of Esbilac (PatAg, Inc., Hampshire, III.).

In a series of experiments reported in detail elsewhere, germfree Beagle puppies were infected with either *H. pylori* or *H. felis* [19–20]. In each experiment there were five infected pups in an isolator and two uninoculated control animals. For 3 weeks post infection the infected and uninfected animals were kept apart. However at 3 weeks, the pups were all mixed so that the uninfected and infected pups could intermingle and play. One week after being in close contact the animals were euthanatized. Thus, in effect a small transmission experiment was undertaken although the major purpose was to follow colonization of gnotobiotic dogs with *Helicobacter* species. The transmission results of this small experiment are reported here (Table 4) because the results are very relevant to the topic under discussion. Despite the relatively brief contact period, *H. pylori* transmitted to both control uninoculated puppies. Also, in contrast to the mouse experiment where transmission of *H. felis* never occurred, one of the two uninoculated pups

Table 4. *Transmission of* Helicobacter *species between infected and uninfected gnotobiotic Beagle puppies*

Inoculated bacterium	Number of puppies positive for the inoculated *Helicobacter* species	
	Inoculated animals	Uninoculated close contact animals
Helicobacter pylori	5/5	2/2
Helicobacter felis	5/5	1/2

Table 5. *Infectivity for mice of gastric mucus and rectal content from a cat infected with a* 'Gastrospirillum'*-like bacterium*

Inoculum	Number of mice showing gastric colonization following inoculation	
	Tested at 1 month	Tested at 2 months
Gastric mucus	5/5	5/5
Rectal content	0/5	0/5
Saline	0/5	0/5

was found to be infected with low numbers of *H. felis* showing that transmission had occurred.

Inoculation of mice with gastric mucus or rectal contents from a cat naturally infected with a 'Gastrospirillum' *– like bacterium*

Cats are naturally infected with gastric spirilla which are present in the gastric tissue and gastric mucus in large numbers. These bacteria are probably a mixture of '*Gastrospirillum hominis*' and *H. felis* with '*Gastrospirillum*' being the dominant organism. The aim of this final experiment was to compare the infectivity of gastric mucus compared with rectal contents in UNSW-SPF mice.

A normal adult cat that had not been on antibiotics was killed with Lethobarb™ at the completion of a physiological experiment. The stomach and rectum of the cat were removed. The stomach contents were discarded and the mucosa lightly washed in normal saline and mucus was scraped and homogenized in saline. The homogenate contained very large numbers of motile '*Gastrospirillum*'-like spiral bacteria. A small amount (1 gm) of the rectal contents from this same animal was also homogenized in saline. Two groups of 10 adult UNSW-SPF mice were intragastrically inoculated with 0·2 ml of either the gastric or rectal homogenate. Ten uninoculated animals served as controls. Five animals from each group were examined 1 month after inoculation and the remaining animals were examined a month later.

Successful colonization of the mouse gastric tissue was assessed by examining gastric biopsies for urease activity and gastric scrapings for the presence of organisms by phase contrast microscopy.

Results are shown in Table 5. The gastric mucus from the spirillum-infected cat was highly infectious and all mouse stomachs were heavily colonized with spirilla. In contrast, none of the mice inoculated with large bowel content from the same cat was infected.

DISCUSSION

The experiments reported above show that *H. felis* is not spread from infected to uninfected mice despite very close contact. Since mice are coprophagous by nature it is likely that during the 8 weeks of contact, uninfected mice would ingest the faecal pellets of infected animals. Thus it is reasonable to conclude that the bacterium is not spread via the faeces of infected animals. *H. felis* infected germfree mice exhibit many analogies to human infection with *H. pylori*. As reported previously, these same mice were primarily colonized in the antrum of the stomach. The chronic gastritis with neutrophil activity that developed was similar to that commonly observed in the human. Also like humans, the helicobacter could not be grown from the stools of infected animals. As *H. felis* forms cocci like *H. pylori*, the contact uninoculated mice should have become infected if these forms were non-culturable but viable. The non-infectious nature of stools from animals with gastric infection was further demonstrated by the experiment with the feline gastric mucus and rectal contents. Clearly the gastric spirilla lose viability as they pass down the gastrointestinal tract. In all gnotobiotic work done to date with either *H. felis* or *H. pylori* the same has been found. That is, intestinal content contained no viable helicobacters even though the stomach is heavily colonized. This has been shown in piglets, [21–22], dogs [19–20], mice [16], and rats [17]. It has been suggested that the reason that *H. pylori* cannot be grown from humans faeces is the competitive effect of the normal flora, [9]. However, as gnotobiotes lack competitive flora then the helicobacters would pass down the gut unimpeded. As anyone who has worked with mono-associated gnotobiotes knows, overwhelming growth is usually seen throughout the gut due to this lack of competition. Thus, the reason for an absence of *Helicobacter* species in the faeces of infected hosts is independent of gut microflora and is probably due to the fact that these bacteria find it to be an hostile environment due to antimicrobial substances such as bile. If *Helicobacter* spp. are not in faeces how does transmission occur? Certainly there is no doubt that person to person spread does occur. The limited experiments with the gnotobiotic puppies reported here suggests the origin of infective bacteria as, in contrast to mice, transmission did occur between infected and uninfected pups. There is a major functional and behavioural difference between mice and Beagle puppies. Firstly, rodents rarely exhibit the close oral contact of puppies. Also rodents do not have a vomit reflex, unlike pups which are easily stimulated to regurgitate gastric contents. Thus one explanation for the transmission between pups is that the pups regurgitate helicobacter-containing gastric mucus or content which is then passed from dog to dog via oral-oral spread. Our hypothesis is that *Helicobacter* spp. are spread from animal to animal or human to human via regurgitated gastric secretions such as vomitus or refluxed gastric mucus. Albenque and colleagues have suggested that *H. pylori* is passed from mother to child in some African cultures via pre-chewed food balls that mothers feed their children [23]. If the origin is via gastric material then it should be possible to find *H. pylori* in the mouth. However since we suggest transient passage through the mouth rather than oral colonization this will prove difficult to demonstrate. To date there has only been one report of isolation of *H. pylori* from the mouth [24].

Dye and co-workers reported on a patient with gastric metaplasia in the rectum that was colonized with *H. pylori* [25]. Some authors have reported *H. pylori* associated with Meckel's diverticula [26]. To get to these locations in both large and small intestine one would have to accept that some bacteria did pass down the bowel but we would submit this is the rare occasion and that faeces are not the normal method of transmitting helicobacters.

What then of the Graham hypothesis? There is no doubt that the correlations he describes are genuine. There is a strong correlation with *H. pylori* infection in developing countries and infection with proven faecal pathogens such as HAV. However, the same would also be true with respiratory pathogens. Gorbach (personal communication) has commented on the high incidence of pneumonia in persons living in unhygienic conditions. The Australian aboriginal living in depressed conditions has a high rate of faecal-oral spread of pathogens but also has a major problem with respiratory pathogens that are clearly not spread via the faecal-oral route. Recent studies in central America have implicated poor water supplies as a source of *H. pylori* infection and this was taken to indicate faecal contamination, [27]. We do not dispute that this organism can survive for long periods in contaminated water [9]. What we would challenge is the assumption that the water was contaminated with faeces; it could equally have been contaminated with spittle, vomitus or saliva.

If one looks closely at the person to person transmission data then the relative slowness of acquisition in some situations argues against faecal-oral spread. Thus, in a study similar to our serological investigation of the incidence of *H. pylori* in inmates of homes for the intellectually disabled [5]. John Lambert's group showed that the rate of acquisition of the organism by children is 4% per year [27]. This is much slower than would be expected for a traditional faecal-oral pathogen.

The final evidence against the faecal-oral route is more speculative but is worth stating. '*Gastrospirillum hominis*' is most likely transmitted to human patients either from dogs or cats. If this organism were infective *via* faeces would not a higher prevalence of infection with this organism be observed in developing countries? To date high infection rates with this organism has not been reported in any country. Our experiments indicate that a human strain of the organism can infect mice very well but it will not transmit *via* the faeces. In contrast, the natural yeast of the murine stomach *Torulopsis* will transmit from animal to animal. Likewise the *Gastrospirillum*-like bacterium seen in cats would readily transmit to mice *via* gastric mucus but not *via* faeces.

For a successful intervention of any disease a clear idea of the most common route of transmission is needed. At present the majority of reviews on this topic claim that the route for *H. pylori* is faecal-oral spread. We challenge that view and submit the case is 'not proven'. Workers in the field of *H. pylori* should keep an open mind on this issue as they review the epidemiological data, which may support or refute either the faecal-oral or oral-oral route more strongly than the present evidence.

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